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Urinary calcium is a determinant of bone mineral density in elderly men participating in the InCHIANTI study

Giuseppe Vezzoli, Laura Soldati, Teresa Arcidiacono, Annalisa Terranegra, Rita Biasion, Cosimo Roberto Russo, Fulvio Lauretani, Stefania Bandinelli, Benedetta Bartali, Antonio Cherubini, Daniele Cusi, and Luigi Ferrucci

Division of Nephrology Dialysis and Hypertension, IRCCS San Raffaele Hospital, Postgraduate School of Nephrology, Vita Salute University, Milan, Italy; Department of Sciences and Biomedical Technologies, Nephrology, University of Milan, Milan, Italy; Laboratory of Clinical Epidemiology, Geriatric Department, National Institute of Research and Care on Aging (INRCA), Florence, Italy; Gerontology and Geriatrics, University of Perugia, Perugia, Italy; and Longitudinal Studies Section, Clinical Research Branch, NIA-NIH, Baltimore, Maryland

Urinary calcium is a determinant of bone mineral density in elderly men participating in the InCHIANTI study.

Background. It is generally acknowledged that calcium excretion is a determinant of bone mineral density. Since data confirming this hypothesis are not conclusive, the present study evaluates the relationship between calcium excretion and volumetric bone mineral density (vBMD) in a sample of general population mostly composed of elderly subjects.

Methods. This relationship was studied in 595 subjects in good health (M/F 302/293), selected from the InCHIANTI population, an epidemiologic survey on aging in Tuscany (Italy). Of these subjects, 432 (72.6%) were 65 years old or older. Trabecular and cortical apparent vBMDs were measured by peripheral quantitative computed tomography at right tibia and standardized to age and body mass index (BMI) in each gender (z-score).

Results. Men in the highest tertile of calcium excretion had significantly lower trabecular vBMD, and were more likely to have a trabecular z-score of −1 or less. These results were confirmed in men older than 64 years, but not in women and younger men. Sodium excretion and 25-hydroxycolecalciferol (25(OH)D) were greater in men and women in the highest tertile. No differences among tertiles were observed for cortical vBMD, circulating levels of interleukin-1β and interleukin-6, and intake of principal nutrients and calcium. The lower levels of vBMD z-score were confirmed in men in the highest tertile of calcium excretion, standardized to creatinine clearance, sodium excretion, plasma calcium, and logarithm of circulating 25(OH)D, and resulted to be associated with calcium excretion at multiple regression analysis in men.

Conclusion. High calcium excretion is associated with a decreased trabecular BMD in elderly men and may predispose men to trabecular bone loss.

Key words: bone mineral density, urinary calcium excretion, cytokines, hypercalciuria, calcium intake.

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Osteoporosis is a chronic and potentially invalidating disorder that negatively influences bone strength, determining a high risk of bone fractures. Osteoporosis typically affects postmenopausal or elderly women, but may also occur in premenopausal women and men at different ages [1–3]. Bone mass decline in women is accelerated by the privation of the protective activity provided by estrogens [4], but also other factors, like vitamin D deficiency with secondary hyperparathyroidism, inadequate dietary calcium intake, or polymorphisms of specific genes [5, 6], may influence bone mass preservation, predisposing to osteoporosis. These defects may also contribute to bone mass loss in men, but the specific causes for male osteoporosis remain still unclear [2, 3, 7]. In keeping with these findings, osteoporosis is considered as a complex disease with a multifactorial pathogenesis [2, 6].

Elevated calcium excretion is generally included among the factors predisposing to osteoporosis [2, 7, 8], but scarce information is available about the relationship between urinary calcium excretion and bone mineral density (BMD) in general healthy population. This point has been mainly explored in osteoporotic or stone-forming patients with primary hypercalciuria, a defect characterized by high calcium excretion without any apparent alterations justifying its presence. Primary hypercalciuria can be detected in 19% of osteoporotic postmenopausal women [9] or men [7, 8], while it is observed in 5% to 10% of the general population. Among calcium stone-forming patients primary hypercalciuria is present in 40% to 50% of cases. Low values of BMD and increased risk of bone fractures have been observed in hypercalciuric stone formers [10–17]. A progressive bone loss in stone formers could result from a urinary calcium excretion exceeding the amount of calcium absorbed in intestine [11, 18], but could also result from a great ingestion of protein that increases dietary acid load, thus stimulating calcium

release from bones and reducing tubular calcium reabsorption [11, 17, 18]. Furthermore, an increased production of cytokines could enhance calcium excretion and bone loss stimulating osteoclast activity and bone resorption [13–15].

The present work was aimed to explore the relationship between calcium excretion and bone density in an elderly general population. To address this issue, we used data from InCHIANTI, a population-based epidemiologic study on life quality decline in older people, performed in Tuscany, Italy [19]. InCHIANTI is particularly suited for this purpose because participants were 65 years old or older, with the exception of a smaller sample of subjects younger than 65 years. Volumetric BMD (vBMD), estimated by peripheral quantitative computed tomography (pQCT) and 24-hour calcium excretion, were measured in all participants. In addition, nutrient intake and cytokine serum concentrations were estimated [20].

METHODS

Study population

InCHIANTI is an epidemiologic survey performed in 2 Italian towns located in the Chianti countryside [19]: *Greve in Chianti* (11,709 inhabitants; rural area) and *Bagno a Ripoli* (4704 inhabitants; suburban area of Florence). The InCHIANTI population consisted of a random sample of the population aged 65 years or older, living in the 2 catchment areas and selected from the population registries. In addition, 30 men and 30 women were randomly selected in each decade between 20 and 65 years and enrolled in the study population. The participation rate was 69.4% in individuals with age younger than 65 years, and 91.6% in older subjects. The original sample was composed of 1530 subjects, but only 1012 underwent a complete interview, medical examination, and pQCT assessment, and were resulted to have correctly collected 24-hour urine. From this sample, we excluded participants who were or had been under treatment with postmenopausal hormonal replacement therapy ($N = 48$), diuretics ($N = 113$), bisphosphonates ($N = 27$), steroids ($N = 78$), vitamin D metabolites ($N = 19$), chemotherapies ($N = 1$), or other drugs affecting calcium metabolism ($N = 3$). We also excluded participants with cancer ($N = 47$), diabetes ($N = 96$) or other endocrine disorders ($N = 55$), renal failure (plasma creatinine >1.3 mg/dL, $N = 28$), hypercalcemia (plasma calcium >2.60 mmol/L, $N = 9$), chronic hepatitis ($N = 6$), those who had undergone colon or stomach resection ($N = 11$), those in bad general condition due to neurologic disorders or other causes ($N = 29$), and the women with early menopause due to surgical ovarian excision ($N = 29$). After exclusion of these subjects, 595 (M/F 302/293) were admitted to our study: 432 older than 64 years (M/F 219/213), and 163 younger than 65 years (M/F 83/80).

Participants were divided in 3 percentile groups (tertiles) according to urinary calcium excretion in each gender. Individuals below the 33.3rd percentiles were assigned to the lowest tertile of calcium excretion (tertile 1; M/F 100/98), those between the 33.3rd and 66.6th percentile to the middle tertile of calcium excretion (tertile 2; M/F 101/98), and those above the 66.6th percentile to the highest tertile of calcium excretion (tertile 3; M/F 101/97).

Participants were considered hypercalciuric when their 24-hour calcium excretion was greater than 7.5 mmol in men or 6.25 mmol in women, or greater than 100 μmol/kg of body weight independent of gender [21].

The InCHIANTI study protocol was approved by the ethical committee of National Institute of Research and Care on Aging in Florence. All subjects received an extensive description of the purposes and known risks of the study procedures; all gave their informed consent.

Methods

Participants received a prestructured interview, a medical and functional examination, and extensive testing. At the end of the interview, the interviewers explained to the participants and their relatives the correct method for the 24-hour urine collection, and provided a large plastic bottle containing 1 g of boric acid as preservative. The participants were instructed to collect in a bottle all the urine produced in the following 24 hours, and to make the maximum effort to avoid dispersing urine during the collection period. Time of start and end of urine collection and episodes of urine dispersion had to be annotated in a diary. Participants carried the entire container to the study clinic and were immediately questioned about possible problems encountered during the collection, and any comment was reported in the database. The urine volume was measured, and 7 aliquots of 10 mL were stored at −80°C. Only samples from subjects who reported no episodes of dispersion were taken into account for this analysis. Height and weight were measured in each participant, and body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m).

Creatinine, calcium, and sodium excretions were measured in 24-hour urine. Calcium, parathyroid hormone (PTH), 25-hydroxycolecalciferol (25(OH)D), and creatinine concentrations were measured in plasma. N-Telopeptide cross-links of the type I collagen (NTx) were measured in 24-hour urine only in participants from Greve in Chianti (146 men and 148 women).

Serum levels of 25(OH)D were measured by radioimmunoassay (DiaSorin, Inc., Stillwater, MN, USA) after extraction of samples with acetonitrile. Intra- and interassay coefficient of variations (CVs) were 8.1% and 10.2%. Serum intact parathyroid hormone levels (PTH) were measured using a 2-site immunoradiometric assay (N-tact

PTHSP; DiaSorin, Inc.). Intra- and interassay CVs were <3.0 and 5.5%.

Interleukin-6 (IL-6) and interleukin-1β (IL-1β) were quantified in serum with enzyme-linked immunosorbent assay (ELISA) methods (Cytoscreen, human IL-6 and human IL-1β ELISA UltraSensitive; BioSource International, Inc., Camarillo, CA, USA). The interassay CV was 7.0% for all assays. The serum interleukin-6 receptor (IL-6R) level was determined by ELISA method (Cytoscreen, human sIL-6 R; Biosource International, Inc.). The serum level of IL-1 antagonist (IL-1ra) was detected by ELISA method (EASIA ELISA Human IL-1ra; Biosource International, Inc.).

Urinary N-Telopeptide was measured by ELISA method (Osteomark Bouty, Milan, Italy) and normalized to creatinine excretion [nmol of bone collagen equivalent (BCE)/mmol of creatinine (Cr)]. Intra-assay and interassay CVs of the assay were both lower than 8%.

Apparent vBMD was measured by peripheral quantitative computed tomography (pQCT) at the right tibia (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany). The right leg extended was positioned inside the gantry of the pQCT device while the subject was seated. The distal end of the tibia (the tibio-talar joint cleft) was identified using a pQCT longitudinal scout view, and was used as an anatomic marker for the identification of the measurement sites. The length of the tibia was assessed as the distance between the medial knee joint cleft and the medial malleolus identified by manual palpation in the participant lying supine. Standard 2.5-mm thick transverse scans were obtained at 4% of the tibial length, where trabecular bone is the most represented, and at 38% of the tibial length, where the cortical shell is usually thicker than 2.5 mm, and an accurate detection of bone boundaries is allowed [22]. The pQCT cross-sectional images were analyzed using the BonAlyse software (BonAlyse Oy, Jyvaskyla, Finland), which automatically identifies bone density and geometry in pQCT scans. Different tissues in the analysis were distinguished according to different density thresholds: areas with density values above 710 mg/cm^3 were considered as "cortical bone," while areas with density values between 180 and 710 mg/cm^3 were considered as "trabecular bone" [23]. From the pQCT images we derived: (*1*) trabecular vBMD, as the average apparent density of the trabecular bone area at the 4% site of tibia; (*2*) cortical vBMD, as the apparent volumetric density of cortical bone measured at the 38% site of tibia. Due to the wide range of age and BMI among the participants, these parameters, measured by pQCT device as mg/cm^3, were expressed in the text as residuals standardized for age and BMI in each gender in the whole population (z-score). The population was divided into 2 groups of vBMD, the first with vBMD z-score equal to −1 or lower, the other with z-score greater than −1. The precision error of the XCT2000 is below 1% [23, 24].

Nutritional data were collected using the food-frequency questionnaire originally developed and validated for the assessment of dietary intake in Italian volunteers participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [25]. The questionnaire was organized in 2 parts, the first focusing on general dietary habits, the second investigating the frequency of consumption of specific food items or dishes during the latest year. The EPIC questionnaire was administered by trained interviewers, and was analyzed with software specifically designed for the EPIC-Italy survey [26].

Statistical analysis

Distribution of categorical variables was compared by Fisher exact test or by multinomial logistic regression. The relative risk to have a vBMD z-score equal to −1 or lower was estimated by the calculation of the odds ratio (OR) and 95% confidence intervals (95% CI). Quantitative variables were reported in the text as mean ± standard error. Differences of the means were tested by Mann-Whitney *U* test or one-way analysis of variance (ANOVA) with Bonferroni post-hoc multiple comparisons test. The associations of calcium excretion or vBMD with the other variables were tested by multiple stepwise regression with *R* change evaluation for each variable. Statistical analyses were conducted at the $\alpha = 0.05$ level and were two-tailed. All analyses were performed using the SPSS 10 statistical package (Chicago, IL, USA).

RESULTS

In each gender, participants were divided in tertiles characterized by the highest (range 4.38 to 14 in men and 3.79 to 10.8 mmol/24 hours in women), the middle (range 2.61 to 4.37 in men and 2.15 to 3.76 mmol/24 hours in women), and the lowest calcium excretion (range 0.32 to 2.58 in men and 0.43 to 2.13 mmol/24 hours in women). Findings in tertiles are displayed in Table 1. Although subjects in the highest tertile were younger, trabecular vBMD z-score was lower in men in the highest tertile than in those in the lowest tertile. At the opposite, plasma calcium was greater in men in the highest tertile. Serum 25(OH)D levels were higher in men in the highest than the middle tertile, and in women in the highest than the lowest tertile. Sodium excretion was larger in men and women in the highest tertile. No differences in cortical vBMD, circulating cytokines, and dietary intake of calories, principal nutrients, and calcium were found across tertiles in both genders.

Table 1. Variables (mean values ± SE) measured in men and women across tertiles of 24-hour calcium excretion

	Urinary calcium tertiles			
	Tertile 1	Tertile 2	Tertile 3	All tertiles
Men				
N	100	101	101	302
Age *years*	70 ± 1.6	65 ± 1.7	61 ± 1.6[a]	65 ± 0.9
BMI *kg/m²*	26.4 ± 0.35	27.1 ± 0.31	27.4 ± 0.34	27 ± 0.19
Plasma calcium *mmol/L*	2.32 ± 0.009	2.35 ± 0.009	2.37 ± 0.008[a]	2.35 ± 0.005
Plasma PTH *pg/mL*	23.0 ± 1.09	21.9 ± 0.92	20.3 ± 0.84	21.7 ± 0.55
Serum 25(OH)D *nmol/L*	56.5 ± 3.23	58.3 ± 2.87	68.7 ± 3.29[b]	61.2 ± 1.83
Serum IL-6 *pg/mL*	1.90 ± 0.228	1.83 ± 0.179	1.54 ± 0.136	1.76 ± 0.107
Serum IL-6R *ng/mL*	99.2 ± 5.06	97.6 ± 5.64	109.3 ± 6.15	102.0 ± 3.26
Serum IL-1β *pg/mL*	0.22 ± 0.046	0.21 ± 0.049	0.19 ± 0.034	0.21 ± 0.025
Serum IL-1 ra *pg/mL*	154 ± 10.24	144 ± 10.1	150 ± 10.2	149 ± 5.9
Urinary calcium *mmol/24h*	1.63 ± 0.063	3.36 ± 0.049	6.11 ± 0.186	3.71 ± 0.126
Urinary sodium *mmol/24h*	130 ± 5.3	146 ± 5.7	189 ± 7.7[c]	155 ± 3.9
Urinary NTx *nmol BCE/mmol Cr*	50 ± 5.3 (45)	45 ± 3.0 (53)	46 ± 2.2 (48)	47 ± 2.1 (146)
Trabecular vBMD z-score	0.19 ± 0.103	0.00 ± 0.095	−0.18 ± 0.098[d]	0.00 ± 0.057
Cortical vBMD z-score	0.02 ± 0.112	−0.01 ± 0.089	−0.02 ± 0.097	0.00 ± 0.057
Dietary calories *kcal/24h*	2254 ± 50.1	2421 ± 64.2	2441 ± 61.3	2372 ± 34.2
Calcium intake *mmol/24h*	22.0 ± 0.70	22.1 ± 0.85	21.1 ± 0.8	22.5 ± 0.45
Protein *g/1000 kcal*	37.8 ± 0.46	37.0 ± 0.44	38.0 ± 0.47	37.6 ± 0.26
Lipid *g/1000 kcal*	32.1 ± 0.49	32.5 ± 0.55	32.8 ± 0.53	32.5 ± 0.30
Carbohydrate *g/1000 kcal*	132.2 ± 1.67	126.8 ± 1.64	127.1 ± 1.62	128.7 ± 0.96
Women				
N	98	98	97	293
Age *years*	72 ± 1.4	66 ± 1.7	60 ± 1.6[e]	66 ± 0.9
BMI *kg/m²*	26.5 ± 0.44	26.7 ± 0.39	27.6 ± 0.47	26.9 ± 0.25
Plasma calcium *mmol/L*	2.36 ± 0.009	2.35 ± 0.008	2.37 ± 0.008	2.36 ± 0.005
Plasma PTH *pg/mL*	26.4 ± 1.29	22.9 ± 1.10	21.9 ± 1.11[f]	23.8 ± 0.68
Serum 25(OH)D *nmol/L*	38.9 ± 2.09	46.4 ± 2.9[h]	59.3 ± 4.27[g]	48.2 ± 1.91
Serum IL-6 *pg/mL*	1.39 ± 0.115	1.33 ± 0.140	1.34 ± 0.195	1.38 ± 0.088
Serum IL-6R *ng/mL*	99.3 ± 4.81	107.8 ± 6.85	97.7 ± 5.33	101.6 ± 3.31
Serum IL-1β *pg/mL*	0.20 ± 0.032	0.20 ± 0.033	0.20 ± 0.029	0.28 ± 0.018
Serum IL-1 ra *pg/mL*	139 ± 9.6	131 ± 8.0	157 ± 9.8	142.4 ± 5.30
Urinary calcium *mmol/24h*	1.65 ± 0.067	2.9 ± 0.072	5.43 ± 0.172	3.31 ± 0.111
Urinary sodium *mmol/24h*	111 ± 4.1	128 ± 4.5[i]	159 ± 5.0[c]	133 ± 2.9
Urinary NTx *nmol BCE/mmol Cr*	57 ± 4.4 (45)	65 ± 7.4 (51)	66 ± 6.3 (52)	63 ± 3.6 (148)
Trabecular vBMD z-score	0.05 ± 0.095	−0.08 ± 0.099	0.03 ± 0.108	0.00 ± 0.058
Cortical vBMD z-score	0.04 ± 0.10	−0.03 ± 0.109	−0.01 ± 0.094	0.00 ± 0.058
Dietary calories *kcal/24h*	1808 ± 42.6	1805 ± 60.0	1911 ± 56.2	1841 ± 30.9
Calcium intake *mmol/24h*	20.2 ± 0.81	21.5 ± 1.02	20.5 ± 0.78	20.9 ± 0.51
Protein *g/1000 kcal*	40.6 ± 0.59	39.7 ± 0.51	40.7 ± 0.53	40.3 ± 0.32
Lipid *g/1000 kcal*	35.1 ± 0.52	35.9 ± 0.61	37.4 ± 0.58	36.1 ± 0.33
Carbohydrate *g/1000 kcal*	130 ± 1.70	130.7 ± 1.81	124.7 ± 1.75	128.5 ± 1.02

vBMD was measured by pQCT at right tibia, and was expressed as residuals standardized for age and BMI in each gender (z-score). NTx is N-telopeptide cross-links of the type I collagen; it was measured only in individuals living in Greve in Chianti (their number is between parentheses), and expressed as nmol of bone collagen equivalent (BCE)/mmol of creatinine (Cr).

[a] $P < 0.001$, tertile 3 vs. tertile 1; [b] $P = 0.019$ tertile 3 vs. tertile 2; [c] $P = 0.0001$, tertile 3 vs. the other two tertiles; [d] $P = 0.023$, tertile 3 vs. tertile 1; [e] $P = 0.0001$ tertile 3 vs. tertile 1 and 2; [f] $P = 0.021$ tertile 3 vs. tertile 1; [g] $P = 0.001$ tertile 3 vs. tertile 1; [h] $P = 0.013$ tertile 2 vs. tertile 1; [i] $P = 0.025$ tertile 2 vs. tertile 1 (one-way ANOVA with Bonferroni post-hoc test).

Considering men older than 64 years (Table 2), trabecular vBMD z-score and plasma PTH were lower, while plasma calcium and sodium excretion were greater in the highest tertile. No differences in trabecular vBMD were found across tertiles in women older than 64 years [0.04 ± 0.100 in the lowest ($N = 87$), −0.11 ± 0.121 in the middle ($N = 73$), and 0.06 ± 0.154 in the highest tertile ($N = 53$)] and in men or women younger than 65 years.

The percentage of individuals with trabecular vBMD z-score of −1 or lower (Fig. 1) was increased in the highest tertile, in men taken as a whole (OR 3.2, CI 95% 1.4 to 7.6, $P = 0.008$, multinomial logistic regression with the lowest tertile as the reference group) or aged 65 years or older (OR 4, 95% CI 1.5 to 11, $P = 0.007$). Women with trabecular vBMD z-score of −1 or less were slightly more frequent in the highest tertile in women aged 65 or older (OR 2.6, 95% CI 1.6 to 9, $P = 0.059$).

Twenty-one men (7.0%; age 63 ± 2.9, BMI 27.9 ± 0.91 kg/m²) and 25 women (8.5%; age 60 ± 3.3 years, BMI 27 ± 1.05 kg/m²) were hypercalciuric. All hypercalciurics were in the highest tertile. Trabecular vBMD of −1 or less was detected in 7 (33.3%) hypercalciuric and 35 (12.5%) normocalciuric men ($P = 0.016$, Fisher exact test; OR 3.5, 95% CI 1.3–9.3), and in 6 (24%) hypercalciuric and 31 (11.6%) normocalciuric women ($P = 0.106$).

Table 2. Variables (mean values ± SE) across tertiles of 24 hour calcium excretion in men of 65 years or older

	Urinary calcium tertiles			
	Tertile 1	Tertile 2	Tertile 3	All tertiles
N	80	74	65	219
Age *years*	76 ± 0.9	73 ± 0.6[b]	71 ± 0.6[a]	74 ± 0.4
BMI kg/m^2	26.6 ± 0.22	27.3 ± 0.35	27.4 ± 0.40	27.1 ± 0.22
Plasma calcium *mmol/L*	2.31 ± 0.009	2.34 ± 0.010	2.37 ± 0.009[c]	2.34 ± 0.006
Plasma PTH *pg/mL*	23.5 ± 1.28	22.5 ± 1.11	19.4 ± 1.0[d]	22.0 ± 0.68
Serum 25(OH)D *nmol/L*	55.5 ± 3.55	56.3 ± 3.37	66.9 ± 3.71	59.1 ± 2.07
Serum IL-6 *pg/mL*	1.56 ± 0.072	3.36 ± 0.058	6.13 ± 0.257	3.52 ± 0.150
Serum IL-6R *ng/mL*	127 ± 6.1	147 ± 6.8	186 ± 10.2[e]	151 ± 4.7
Serum IL-1β *pg/mL*	50 ± 6.3 (35)	46 ± 3.5 (37)	48 ± 2.8 (33)	48 ± 2.6 (105)
Serum IL-1 ra *pg/mL*	0.22 ± 0.120	−0.0 ± 0.117	−0.29 ± 0.127[f]	−0.01 ± 0.071
Urinary calcium *mmol/24h*	−0.03 ± 0.135	0.01 ± 0.104	−0.12 ± 0.130	−0.01 ± 0.072

[a] $P = 0.0001$, tertile 3 vs. tertile 1; [b] $P = 0.031$ tertile 2 vs. tertile 1; [c] $P = 0.0001$, tertile 1 vs. tertile 3 and $P = 0.036$, tertile 3 vs. tertile 2; [d] $P = 0.042$ tertile 3 vs. tertile 1; [e] $P = 0.0001$ tertile 3 vs. tertile 1 and $P = 0.002$ tertile 3 vs. tertile 2; [f] $P = 0.012$ tertile 3 vs. tertile 1.

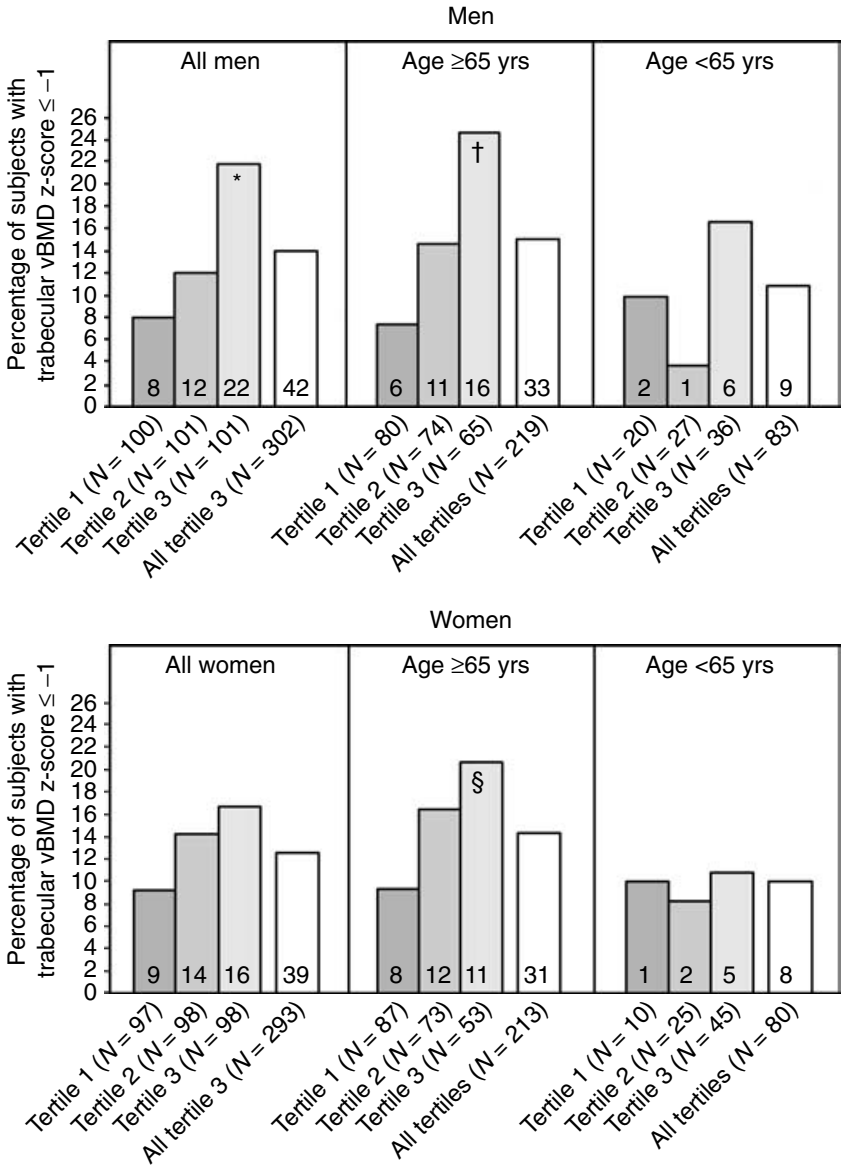

Fig. 1. Distribution of men with trabecular z-score of −1 or less across tertiles of calcium excretion. Tertile 1 is the lowest tertile, tertile 2 the middle, and tertile 3 the highest. vBMD was measured by pQCT at right tibia, and was expressed as residuals standardized for age and BMI in each genders (z-score). The number of subjects is reported at the base of each column. *OR 3.2, 95% CI 1.4–7.6, $P = 0.008$, †OR 4, 95% CI 1.5–11, $P = 0.007$, §OR 2.6, 95% CI 1–6.9, $P = 0.059$ (multinomial logistic regression, tertile 1 as the reference group).

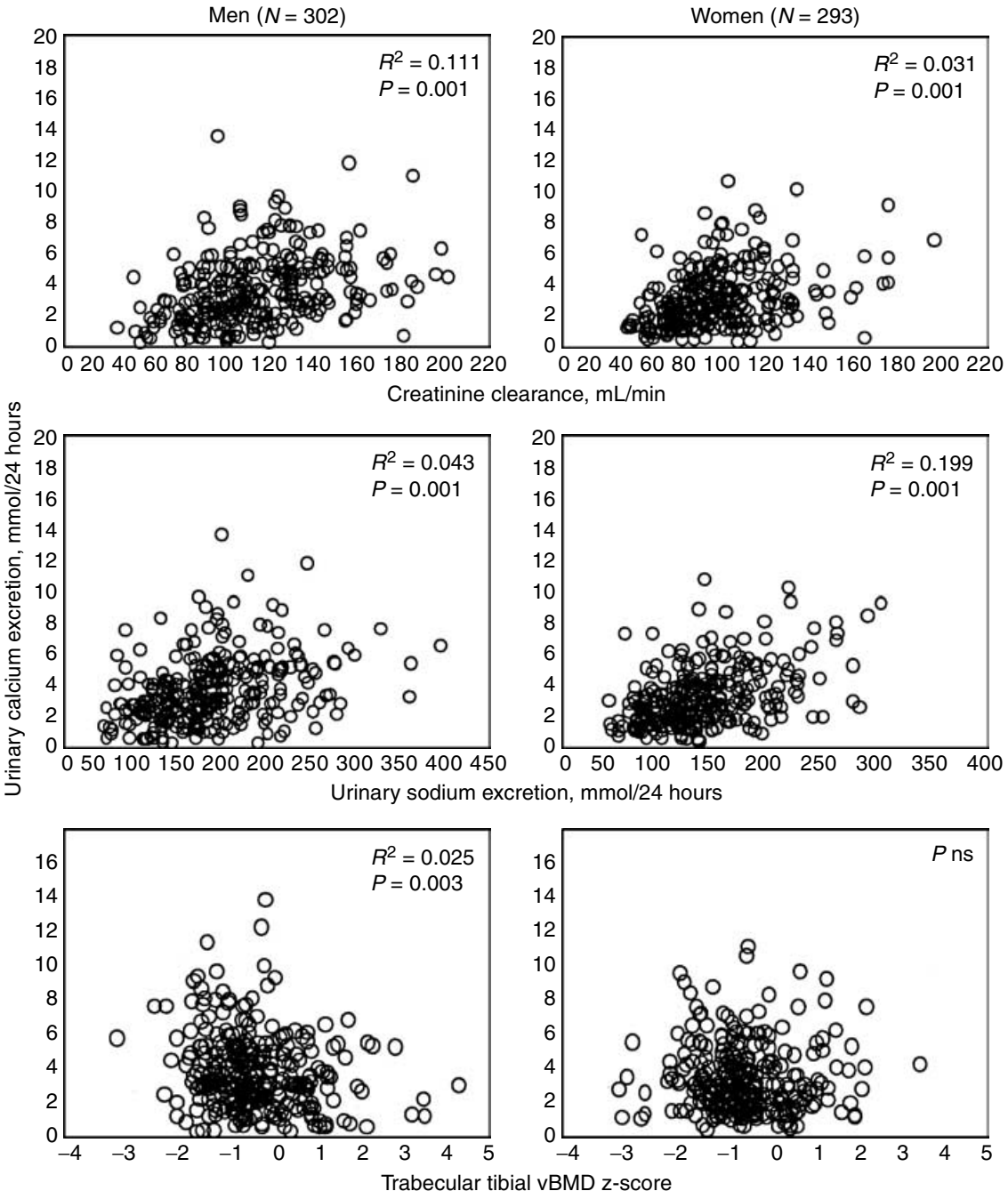

Fig. 2. Relationships of urinary calcium excretion with creatinine clearance, urinary sodium, and trabecular vBMD in men and women. vBMD was measured by pQCT at right tibia, and was expressed as residuals standardized for age and BMI in each genders (z-score). Results of multiple stepwise regression (R^2 change and significance) are reported for each relationship.

Variables associated with calcium excretion were analyzed by multiple stepwise regression in each gender. Age, BMI, plasma concentrations of calcium, PTH, IL-6 and IL-1β, logarithm of plasma 25(OH)D, creatinine clearance, sodium excretion, trabecular tibial vBMD z-score, dietary intake of calcium, protein, carbohydrate, and lipid were independent variables in the model. In men, creatinine clearance, sodium excretion, plasma calcium concentration, trabecular z-score, and logarithm of circulating 25(OH)D were associated with calcium excretion (Table 3). In women, sodium excretion and creatinine clearance were associated with calcium excretion (Table 3). In order to circumvent possible confounding effect of the variables indicated by multiple regression analysis, the relationship between calcium excretion and bone density was tested after standardization of calcium

Table 3. Variables associated to calcium excretion (mmol/24h) in stepwise multiple regression model

Variable	R^2 change	R^2 change P	B	P
Men				
Creatinine clearance	0.111	0.001	0.017	0.0001
Urinary sodium	0.043	0.001	0.006	0.001
Plasma calcium	0.029	0.002	0.984	0.003
Trabecular BMD z-score	0.025	0.003	−0.352	0.004
Logarithm of plasma 25(OH)D	0.013	0.035	1.131	0.035
Women				
Urinary sodium	0.199	0.001	0.014	0.0001
Creatinine clearance	0.031	0.001	0.013	0.001

Age, BMI, plasma concentrations of calcium, PTH, IL-6 and IL1β, logarithm of plasma 25(OH)D, sodium excretion, trabecular tibial vBMD z-score, dietary intake of calcium, protein, carbohydrate, and lipid as independent variables. Analysis was conducted in each gender separately (260 men and 248 women).

excretion to sodium excretion, creatinine clearance, plasma calcium, and logarithm of circulating 25(OH)D in men. vBMD not being considered in the model, the relative risk of vBMD reduction was calculated in tertiles of standardized calcium excretion. Ranges were 0.22 to 6.48 in the highest ($N = 100$; age 66 ± 1.4; BMI 27.3 ± 0.34 kg/m^2), −0.44 to 0.2 in the middle ($N = 101$; age 65 ± 1.7; BMI 27.0 ± 0.31 kg/m^2), and −2.04 to −0.45 in the lowest tertile ($N = 101$; age 64 ± 1.8; BMI 26.7 ± 0.35 kg/m^2). Trabecular vBMD z-score was lower in the highest tertile of calcium excretion residuals, both in the whole group of men (–0.23 ± 0.101 in the highest tertile, 0.12 ± 0.073 in the middle tertile, and 0.22 ± 0.105 in the lowest tertile; $P = 0.004$, the highest vs. the lowest tertile; one-way ANOVA with Bonferroni post-hoc test), and in men aged 65 years or older (−0.34 ± 0.121 in the highest tertile, 0.04 ± 0.107 in the middle tertile, and 0.23 ± 0.142 in the lowest tertile; $P = 0.004$, the highest vs. the lowest tertile). Men with trabecular vBMD z-score of −1 or less were more represented in the highest tertile: they were 8 (7.9%) in the lowest, 11 (10.9%) in the middle, and 23 (23%) in the highest tertile (OR 3.5, 95% CI 1.5–8.2, $P = 0.005$; multinomial logistic regression, the lowest tertile as the reference group).

Variables associated with vBMD were tested in each gender by a multiple stepwise regression model considering urinary NTx, urinary calcium, and the other factors used for calcium excretion analysis as independent variables. In men ($N = 146$), urinary NTx (R^2 change = 0.037, $P = 0.022$; B = −0.006, $P = 0.023$), calcium excretion (R^2 change = 0.039, $P = 0.019$; B = −0.108, $P = 0.007$), and age (R^2 change = 0.029, $P = 0.038$; B = −0.009, $P = 0.038$) were associated with trabecular vBMD. In women ($N = 148$), urinary NTx (R^2 change = 0.036, $P = 0.027$; B = −0.005, $P = 0.009$) and IL-6 (R^2 change = 0.04, $P = 0.017$; B = 0.109, $P = 0.017$) were found to be associated.

History of hip fractures was positive in 3 men and 3 females. Four of them presented trabecular z-score of −1 or less, and 2 of them z-score greater than −1 (OR 13.7, 95% CI 2.5–76, $P = 0.004$; Fisher exact test). None of them was in the lowest tertile of calcium excretion.

DISCUSSION

The relevance of calcium excretion for the preservation of bone density in men is suggested by our analysis of participants in InCHIANTI, an Italian epidemiologic survey on aging. Our findings suggest that high urinary calcium excretion accelerates the progressive age-related bone loss in men, without substantial influence on peak bone mass reached during growth. The negative effect of calcium excretion was slight in women, probably because other factors, like estrogen or vitamin D, are proportionally more important than calcium excretion for bone preservation and for the pathogenesis of osteoporosis [4, 5]. Lack of estrogens is the most effective determinant of BMD reduction in postmenopausal women, and its effect is not directly dependent from calcium excretion [2]. The levels of 25(OH)D suggest that a vitamin D deficit is more frequent in InCHIANTI women than men, especially among women in the lowest tertile of calcium excretion, in whom it may favor a higher PTH secretion in comparison with the other tertiles and a probable reduction of intestinal calcium absorption [27]. This condition may negatively influence vBMD, thus contributing to make us unable to find a negative association between vBMD and calcium excretion in women [27].

In spite of these results, the number of hip fractures was not clearly associated with calcium excretion, but the count of subjects with prevalent hip fractures was too low to make it easy to detect any significant association between these variables.

Different from trabecular bone, cortical vBMD was not related with urinary calcium in our analysis, probably because the process of endostal resorption reduces the thickness of cortical bone without detectable modification of cortical vBMD [28]. This agrees with previous observations, showing that in hypercalciuric subjects demineralization is more remarkable at bone sites where trabecular bone is more represented [11, 12, 17]. Furthermore, we have to consider that pQCT is an accurate method for the analysis of tibial trabecular bone, but its accuracy decreases in tibial cortical bone analysis, thus contributing to explain the lack of relationship between cortical BMD and calcium excretion [29].

The low plasma values of PTH and the elevated plasma concentrations of calcium and 25(OH)D in subjects in the highest tertile of calcium excretion suggest that urinary calcium commonly increases as a result of a more active calcium intestinal absorption, probably stimulated by vitamin D [30]. The increase of serum concentrations of 25(OH)D in InCHIANTI participants with high calcium excretion is likely to be explained by larger vitamin D store [31], and may be associated with increased renal $1.25(OH)_2D$ synthesis [16, 32]. This association may be observed in hypercalciuric stone formers, and has been attributed to an abnormal renal 25(OH)D hydroxylation activity [16, 33]. Production of $1.25(OH)_2D$ may also depend on renal volume, and this may explain the positive relationship of calcium excretion with creatinine clearance [33]. However, circulating $1.25(OH)_2D$ was not measured in InCHIANTI population due to the normal levels of $1.25(OH)_2D$ we previously found in hypercalciuric stone formers [34]. Although 100-fold less active than $1.25(OH)_2D$, 25(OH)D may have a biological activity due to its 1000-fold higher serum concentration. The rise of 25(OH)D may enhance calcium excretion and blood calcium concentrations, and may have a protective effect on BMD as a result of the negative relationship between serum levels of 25(OH)D and PTH [27]. In spite of this setting, vBMD was low in men with high calcium excretion, similar to that previously reported in absorptive hypercalciuria [11, 12, 16]. It may be attributed to a negative calcium balance induced by kidney inability to decrease calcium excretion during fasting or low calcium diet [11, 18, 35]. This especially occurs when diet is poor of milk and dairy products, so that calcium intake falls under 5 mmol a day [11, 30]. In addition, quantitative or qualitative differences in sodium, carbohydrate, protein, or lipid intake can influence calcium absorption and bone calcium metabolism [32, 36–38]. Among them, only sodium ingestion, estimated by us with the measurement of urinary sodium excretion, is increased in InCHIANTI subjects with high calcium excretion. Sodium intake can influence calcium metabolism at different levels. The tubular sodium load following dietary sodium ingestion can decrease tubular calcium reabsorption; thus, it may favor a reduction of BMD when persistent in time [11, 18]. However, sodium-induced calcium excretion may also stimulate $1.25(OH)_2D$ synthesis and intestinal calcium absorption with a positive effect on BMD [36]. In addition, it may be that dietary sodium load promotes intestinal absorption of calcium by stimulating its transport across mucosa. Therefore, whereas sodium intake appears as a determinant of calcium excretion, its consequences on BMD probably change in relation to the characteristics of dietary habits, intestinal absorption, and tubular reabsorption capability in single individuals. In agreement with this hypothesis, no relationships between BMD and sodium excretion were observed in InCHIANTI population [38]. In the light of our results, intake of calcium or other nutrients does not appear able to sustain the reduction of BMD in subjects with high calcium excretion, even though daily balance between excreted and absorbed calcium may be more frequently negative in them.

Alternatively, the reduction of bone density may be attributed to a mechanism in which the defect responsible of high calcium excretion directly involves bone tissue and predisposes to bone loss [12, 16, 39]. An increased production of cytokines may lead to osteoclast activation, bone loss, and urinary calcium wastage. Evidence of this was the more active production of IL-1β by monocytes cultured from stone-forming patients with fasting hypercalciuria [13, 14]. Controversial results were instead obtained about IL-6, whereas an alteration of cytokine synthesis was not observed in absorptive hypercalciuria [13–15]. We did not find any differences of blood concentrations of cytokines, IL-6R and IL-1ra, in individuals with different calcium excretion or vBMD, even though the levels of IL-6 appeared to be associated to vBMD in women. However, we did not identify subjects with fasting hypercalciuria; moreover, circulating levels of IL-1β and IL-6 could not be representative of the amount of cytokines produced by osteoblasts in their remodeling activity.

CONCLUSION

Findings in InCHIANTI display that BMD is more frequently reduced in men with high calcium excretion, though they were not necessarily hypercalciuric. Although we cannot exclude the role of cytokines or negative calcium balance, a specific and probably genetic defect of calcium metabolism may be expressed in bones, as well as in other calcium-handling tissues, and may favor bone demineralization in subjects with high calcium excretion [40]. To our knowledge, this is the first population-based study in which calcium excretion arises as a determinant of bone mineralization. Early measurements of calcium excretion could help to detect men predisposed to losing bone mass and to developing osteoporosis.

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Reprint requests to Dr. Giuseppe Vezzoli, Unità di Nefrologia Dialisi e Ipertensione, IRCCS Ospedale San Raffaele, via Olgettina 60, 20132, Milano, Italy.
E-mail: vezzoli.giuseppe@hsr.it

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